

*Antitumor/antiviral drugs target on STING*

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STING (Stimulator of Interferon Genes, also known as ERIS, MITA and MPYS) is an important adaptor protein in innate immunity. It plays a key role in cytosolic DNA mediated IFN production. STING is the direct sensor of cyclic dinucleotides (CDN) in cytosol, including c-di-GMP from pathogen and cGAMP synthesized by cGAS after DNA stimulating. After binding to and activated by CDNs, STING recruits and activates TBK1 and IRF3 by its C terminal tail. IRF3 is phosphorylated and then dimerize to enter nucleus, resulting in type I interferon (IFN) production.

5,6-dimethylxanthenone-4-acetic acid (DMXAA) and 10-carboxymethyl-9-acridanone (CMA) were identified as antitumor/antiviral compounds which acted very well in mouse model but failed in human clinical trial. Further research showed that DMXAA and CMA bind to and activate mouse STING, but not human STING. To further investigate the mechanism of ligands' species selectivity, we studied on human STING (hSTING), rat STING (rSTING) and mouse STING (mSTING) by functional and structural analysis. We found that human and rat STINGs display more similar signaling profiles toward DMXAA and CMA than that of human and mouse STINGs, suggesting that rat is more suitable for preclinical testing of STING-targeted drugs.

Previous research showed that a single point mutation in hSTING S162A renders hSTING sensitive to DMXAA. Through molecular dynamics simulations, we revealed how this single mutation alter the DMXAA-STING interaction. Compared to mutated systems, structural correlations in the interaction of STING with DMXAA are stronger, and the correlations are cross-protomers in the dimeric protein. Analyses on correlation coefficients lead to the identification of two key interactions that mediate the strong cross-protomer correlation in the DMXAA-STING interaction network: DMXAA-267T-162S\* and 238R-260E\*. These two interactions are partially and totally interrupted by the S162A and E260I mutations, respectively. Moreover, a smaller number of water molecules are displaced upon DMXAA binding to STING than that on binding to its mutants, leading to a larger entropic penalty for the former. Considering the sensitivity of STING and two of its mutants to DMXAA, a strong structural correlation appears to discourage DMXAA-STING binding. Such an observation suggests that DMXAA derivatives, which are deprived of hydrogen-bond interaction with both 162S\* and 267T, are potential agonists of human STING.

Taken together, our findings suggest better animal model for STING-targeted drugs and shed light on optimization of hSTING agonist.

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